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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (1): 157-159, 2000

Role of Plant Growth Regulators (Auxin and Cytokinin) in Callus Induction in Rice (*Oryza sativa* L.) C.V. DM-25

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Abstract: Methods were developed to optimize the media for callus induction from mature embryos of indica rice (*Oryza sativa* L.) CV. DM-25. Callusing from seeds of rice was observed with various levels of 2,4-d alone and in combination with different concentratiom of benzyl adenine. With the gradual increase of 2,4-d concentration in callus induction medium, there was gradual increase in callusing frequency.

Key words: Plant, regulators, callus, induction, rice

Introduction

Tissue culture technology had opened new avenues of research and has become an important alternative of conventional propagation procedures for the plants. It also provides innovative breeding technique for the development of crop varieties with desirable traits. This technique is being used as an adjunct to more traditional means in plant modification. The potential for genetic improvements of monocots especially of rice through tissue culture including somatic hybridization has recently been applied (Kyozuka *et al.,* 1987). Callusing has been reported with varying degree of success in rice which has great potential to regenerate into complete plantlets (Heyser *et al*., 1983; Nakano and Maeda, 1979). However, in most of these reports, the formation of shoots and plantlets in rice is rather infrequent and transient. Occurrence of wide spectrum of mutational events during tissue culture, results in genetic variability in co-adapted, agronomically useful cultivars, without the need to resort to hybridization. The manipulation at cell level is easier than at plant level and by employing somatic cell genetic techniques such as protoplast fusions and genetic engineering new crop varieties can be produced and existing ones can be improved successfully. The induction of callus from rice embryo is pre-requisite for initiation of embryogenic cell suspension as a source of protoplasts for transformation and somatic hybridization experiments as well as in vitro selection of disease resistant mutants to elucidate the phenomenon of soma clonal variation.

Materials and Methods

Seeds of indica rice were taken and dehusked manually and were washed with detergent, surface sterilized in 20 percent (V/V) commercial bleach (NaOCl) for 30 minutes with constant shaking. Seeds were washed several times with sterilized distilled water. These explants were placed in jars containing callus induction media with combination of 3 percent sucrose and 0.5, 1.0, 2.0. and 4.0 mg/l 2, 4-D separately. Media were also prepared with 2 mg/l 2,4-D in combination with Benzyl adenine (BA) 0.5, 1.0, 2.09 and 4.0 mg/l respectively. Cultures were incubated in both light and dark conditions.

Data were recorded after two weeks of culturing upto 5 weeks on callus initiation and number of calli produce to computer callus percentage. After 4-5 weeks the developing calli were placed on maintenance media for proliferation.

Results and Discussion

Effect of Auxin concentration for callus initiation: Data collected in relation to callus induction frequency as affected by various levels of 2,4-0 (Auxin) are presented in Table 1 and Fig. 1. Callus induction in relation to dose of 4 mg/l 2,4-D indicated significant superiority over other doses of 2,4-D. Superiority of 4 mg/l 2,4-D could be attributed to higher doses of 2,4-D than lower doses of 2,4-D in other treatments.

From the results it is clear that with the gradual increase of 2,4-D concentration in callus induction medium these was gradual increase in callus initiation frequency. In some cases it was noted that on sub-culturing calli exuded certain phenolic compounds and turned brown. Friable and nodular calli arose from primary calli during subculturing which were both embryogenic and non embryogenic and white to yellow in colour. It is quite possible that genotypic specificity of each explant might be responsible in the control of such developmental changes in callus morphology (Table 3). Similar role of 2,4-D in callus induction has also been observed by (Lupotto, 1984; Mohanty and Ghosh, 1988; Hartke and Lorz, 1989; Zafar *et al.,* 1992).

Effect of Auxin-Cytokinin combinations for callus initiation: Comparison was made between different levels of 2,4-D separately and in combination with BA for callus initiation (Table 2, Fig. 2). From the results it is clear that 2 mg/l 2,4-(control) showed significant superiority for callus induction efficiency over all combinations having various doses of BA in combination with 2,4-D (2 ml/l). It is strange to note that different doses of BA with 2, 4-D behaved in different fashion. As it is obvious from the data that as the BA concentration is increased there was a decrease in

Table 1: Callus initiation percentage in *Oryza sativa* L. (rice) CV-DM 25 As affected by different levels of auxin in MS medium

Auxin	Concentration	Callusing frequency (%)	Remarks growth of (calli)
2, 4-D	0.0	00.00	-
	0.5	40.00	+ +
	1.0	61.67	+ + +
	2.0	78.33	+ + + +
	4.0	88.33	+ + + +

+ + + + = Excellent + + + = Good + + = Fare
\- = No callus + = Poor

Table 2: Callus initiation percentage in *Oryza sativa* L. (rice) CV-DM-25 as affected by different combinations of auxin-cytokinin in MS medium

Auxin-Cytokinin	Combi-nations	Callusing frequency	Remarks (growth of calli)
2-D (mg/l)	BA (mg/l)		
2.0	0.0	78.33	+ + + +
2.0	0.5	58.33	+ +
2.0	1.0	48.33	+ +
2.0	2.0	41.67	+ + +
2.0	4.0	36.67	+ + +

+ + + + = Excellent + + + = Good + + = Fare
\+ = Poor - = No callus

Table 3: Response -of embryo-derived callus cultured on different media

Media	Responses
MS-O	No callus formation
$MS_{0.5}$	Callus yellow, fast growth loose, granular, partially necrotic
MS_1	Fast growing, white, compact
MS_2	Callus pale yellow, loose, granular with rapid growth
MS_4	Callus white, friable, necrotic to some extent, with very rapid growth
$MS_2B_{0.5}$	Callus nodular, white, compact, yellow in colour and with slow growth, highly embryogenic
MS_2B_1	Callus loose, white, later became brown and black to some extent
MS_2B_2	Callus, granular compact & white in colour
MS_2B_4	Callus white to pale yellow in colour with very rapid growth

MSO, $MS_{0.5}$, MS_2 and MS_4 indicate MS basal medium supplemented with 0, 0.5, 1.0, 2.0 and 4.0 mg/l 2, 4-D
$MS_2B_{0.5}$, MS_2B_1, MS_2B_2 and MS_2B_4 indicate MS medium having 2 mg/l 2, 4-D and 0.5, 1.0, 2.0 and 4 mg/l BA

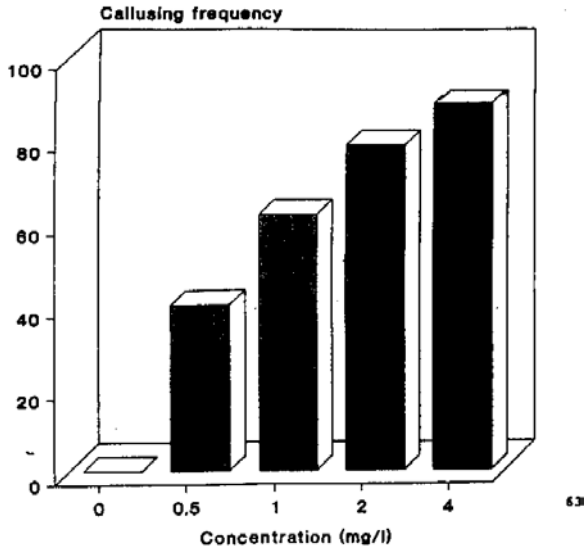

Fig. 1: Callus initiation percentage in *Oryza sativa* L. CV. DM-25 as affected by different levels of auxin

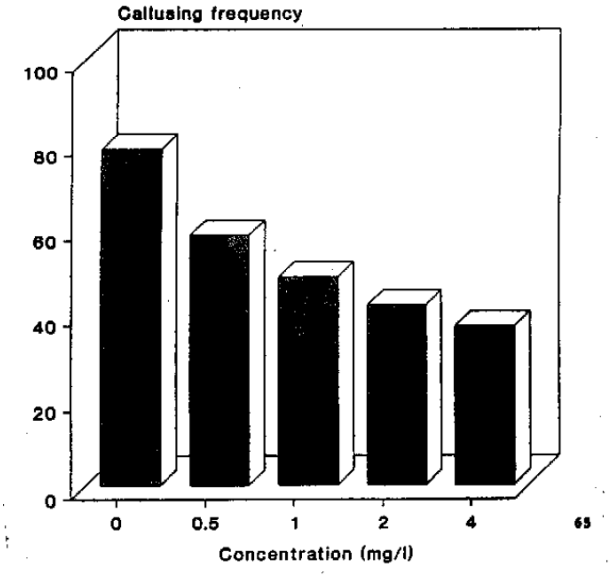

Fig. 2: Callus initiation percentage in *Oryza sativa* L. CV. DM-25 as affected by different combinations of auxin-cytokinin

callus induction frequency. Although callusing frequency was lower in media having both 2, 4-D and BA as compared to media having only 2, 4-D, but the frequency of embryogenic callus formation was higher in the former (Wang and Yan, 1984; Chu *et al*., 1984; Boyes and Vasil, 1984; MacKinnon *et al*., 1986; George and Eapen, 1988), It appears that low level of BA worked better in combination with 2, 4-D than higher doses of BA and any further gradual increase of BA in the media might imparted declining effects.

The range of differences between these doses is rather narrow to pin down the verdict of antagonism or toxicity of PGR at that level. Inferiority of certain doses could also be attributed to the impact of interactions of 2,4-D and BA. May be, the results become prominent if some lower doses are utilized. It is logical that the lower doses of BA favoured rather than producing antagonistic effect with 2, 4-D. Further studies are needed to find out the possibility of having more embryogenic cultures by decreasing BA concentration in combination with 2, 4-D in the callus induction medium.

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